

U.S.S.N. 09/991,152

Filed: November 16, 2001

## RESPONSE TO RESTRICTION REQUIREMENT

## In the Claims

1. (currently amended) A genetically engineered organism selected from the group consisting of bacteria and plants producing polyhydroxyalkanoate, the improvement comprising providing the transgenic organism expressing with a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase so that medium chain length PHA accumulates.
2. (original) The organism of claim 1 further comprising one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.
3. (original) The organism of claim 2 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.
4. (original) The organism of claim 2 wherein the acyl-CoA synthetase is *alkK*.
5. (currently amended) The organism of claim 2 or 3 further expressing a PHA synthase heterologous 3-hydroxyacyl-CoA synthetase activity.
6. (currently amended) The organism of claim 1 ~~or 5~~ further expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.
7. (currently amended) The organism of claim 1 wherein the enzyme is modified to enhance expression in the genetically engineered organism.
8. (currently amended) The organism of claim 5 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

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9. (original) The organism of claim 8 further expressing selectable marker genes, wherein the organism is a whole plant.
10. (currently amended) The organism of claim 5 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.
11. (original) The organism of claim 8 wherein expression of the transgene is targeted to a tissue or organelle selected from the group consisting of seeds, leaf, plastids, and peroxisomes.
12. (original) The organism of claim 10 wherein the bacteria is *E. coli* and PHA accumulates within the bacteria.
13. (currently amended) A method of engineering a PHA biosynthetic pathway in a transgenic organism selected from the group consisting of bacteria and plants which produce polyhydroxyalkanoate, the improvement comprising providing the organism with a construct comprising a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase so that medium chain length PHA accumulates, and making the organism.
14. (original) The method of claim 13 wherein the construct further comprises one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.
15. (currently amended) The method of claim 14 wherein the ~~enzymes have the catalytic activity of~~ construct comprises a transgene encoding a 3-hydroxy acyl-CoA synthetase.

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16. (original) The method of claim 15 wherein the construct further comprises a transgene encoding a PHA synthase.
17. (original) The method of claim 16 wherein the organism is a plant.
18. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.
19. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.
20. (currently amended) A method of making PHA comprising growing a transgenic organism selected from the group consisting of bacteria and plants, the organism producing polyhydroxyalkanoate and expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase.
21. (currently amended) The method of claim 20 wherein the organism further ~~comprising~~ comprises one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.
22. (original) The method of claim 21 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.
23. (original) The method of claim 21 wherein the organism further express a PHA synthase.

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24. (original) The method of claim 22 wherein the organism further express a PHA synthase.
25. (original) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.
26. (original) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.
27. (cancel) A method of screening for enzymes encoding 3-hydroxy acyl ACP thioesterase activities comprising:
- a) coexpressing an enzyme in a heterologous organism that expresses a PHA synthase and a 3-hydroxyacyl-CoA synthetase or a CoA transferase, and
  - b) growing the organism under appropriate conditions for the production of a PHA.
28. (cancel) A method for increasing the levels of C8 and C10 hydroxyacids or fatty acids of a plant oil composition comprising
- a) expressing a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterase, and
  - b) growing the plant under appropriate conditions for the production of the plant oil composition.
29. (cancel) The organism of claim 10 wherein the bacteria is *E. coli*, and

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wherein 3-hydroxy acids are secreted into the culture medium.